Ozonation of Caffeine in Aqueous Solution

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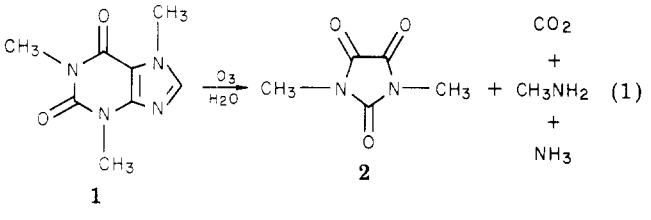
Caffeine (1,3,7-trimethylxanthine), a component of waste water, produces numerous rearranged products upon ozonation. These products include dimethylparabanic acid (**2**), 1,3-dimethyloxonic methylamide (**3**), 1,3-dimethyl-5-azabarbituric acid (**4**), methylparabanic acid (**5**), *N,N'*-dimethyloxalamide (**6**), *N*-methyloxalamide (**7**), 1,3-dimethyl-5-azauracil (**8**), and 1,3-dimethyl-5-hydroxyhydantoin (**9**). The ozonation of 1-ethyl-3,7-dimethylxanthine (**12**) and 1,3-dimethyl-7-ethylxanthine (**13**) yield product mixtures similar to those from caffeine (**1**). The production of ethylated oxonic acid derivatives allows the elucidation of a mechanistic pathway for 1,3-dimethyloxonic methylamide (**3**) formation from caffeine (**1**). The ozonation of trideuteriomethyl-labeled caffeine analogues demonstrates that 86% dimethylparabanic acid (**2**) results from the pyrimidine ring of caffeine (**1**), the rest involving the imidazole ring. The ozonation of 1,3-dimethyluracil (**25**) produces dimethylparabanic acid (**2**) and 1,3-dimethyl-5-hydroxyhydantoin (**9**). The latter compound retains C-5 and expels C-6 as demonstrated by ozonation of deuterium-labeled starting material. The ozonation of 1,3,5-trimethyluracil (**28**) produces *N*-methylpyruvamide (**29**) and 1,3,5-trimethyl-5-hydroxyhydantoin (**30**). The data obtained permit the proposal of reasonable mechanisms for the formation of the major products.

Caffeine, a component of coffee and tea, also appears to be a rather common component in treated domestic waste water.[1] In a preliminary study of the organic compounds present in waste water, we found caffeine to be one of the principal components.[2] Since ozone is being considered in place of chlorine as a disinfectant for waste water, it is desirable to ozonize individual waste water components and identify the products of these reactions. By doing this, the resulting data will facilitate the identification of compounds found in ozonized waste water. This study has clarified the chemistry of caffeine with ozone and provided a model system upon which the ozonation chemistry of nucleic acid bases can be evaluated.

Several studies have been made of caffeine oxidation. For example, in 1890 Leipen treated an aqueous solution of caffeine (**1**) with ozone and reported that four products, dimethylparabanic acid (**2**), methylamine, ammonia, and carbon dioxide, are generated (eq 1).[3] Previously it had



1 2 (1)

been reported that dimethylparabanic acid (**2**) is the stable end product from the oxidation of caffeine under many experimental conditions.[4,5]

We found that caffeine in dilute aqueous solution is destroyed rapidly by ozone and that 10–20 discernible products are generated. Product analysis was conducted by gas chromatography, a method not available to earlier workers. The number and ratio of these products appear to be dependent on experimental parameters such as the presence of cosolvents and metal ions; other parameters such as temperature, concentration, and reaction time appear to have little effect. The results reported in this paper are those from typical experiments in which the caffeine (**1**) concentration was 3.4×10^{-3} M and the reaction time was 90 min at 20 °C. Seven hundred and fifty milliliters of this solution was allowed to react with an ozone–oxygen stream introduced at a flow of 600 mL/min (17 mg of O_3/min). Under these conditions the reaction is complete within 20 min and 4.2 mol of ozone is consumed for each mol of caffeine (**1**). Four of the products constitute about 70% of the reaction mixture. Dimethylparabanic acid (**2**) is indeed a principal product, but, in our hands, it always ranked second in abundance to a component with molecular weight 198. Gas chromatographic retention time (conditions A in the Experimental Section), the product ratio (conditions B), and the apparent molecular weight as determined by mass spectrometry are given in Table I. Figure 1 shows the total ion chromatogram of the product mixture. The mass spectrum of each component is given in Table II.

Table I. Products Formed from Caffeine Ozonation[a]

t_r, min	MW[b]	rel %[c]	t_r, min	MW[b]	rel %[c]
1.0	75		10.1	144	2.7
1.2	59		10.5	157	8.7
2.7	117	6.0	11.7	141	3.4
3.2	116	0.6	12.3	128	5.8
3.6	132	0.1	13.7	210	1.6
4.5	142	23.0	14.9	198	26.4
5.6	102	1.6	15.9	254	2.4
7.0	140	3.6	17.4	226	11.7
8.9	143	2.4			

[a] Gas chromatography-mass spectrometry (GC/MS) was conducted according to conditions A listed in the Experimental Section. [b] Apparent molecular weight as determined by mass spectrometry. [c] Relative percent yield was measured by FID response according to GC conditions B and is not corrected for differences in detector response.

Three major reaction components were isolated, purified, and characterized. Dimethylparabanic acid (**2**) was isolated from a chloroform extract of the aqueous product mixture and purified by recrystallization. Its structure was deduced by spectroscopic techniques and proved by comparison with synthetic dimethylparabanic acid.

The major product of the ozonization (**3**) was isolated by preparative column chromatography on silica gel. The mass spectrum shows an apparent molecular ion (m/e 198)

(1) S. Katz, W. W. Pitt, Jr., C. D. Scott, and A. A. Rosen, *Water Res.*, 6, 1029 (1972).
(2) R. H. Shapiro, K. J. Kolonko, R. T. Binder, R. M. Barkley, G. A. Eiceman, L. P. Haack, and R. E. Sievers, "Proceedings of the 4th Joint Conference on Sensing on Environmental Pollutants" (New Orleans, La., 1978), American Chemical Society, 1978, p 136.
(3) R. Leipen, *Monatsh. Chem.* **10**, 184 (1890).
(4) (a) J. Stenhouse, *Ann. Chem. Pharm.*, **45**, 372 (1843); (b) **46**, 227 (1843); (c) P. Rochleder, *ibid.*, **73**, 57 (1850); (d) R. Maly and F. Hintergger, *Monatsh. Chem.*, **2**, 87 (1881); (e) T. J. Delia and G. B. Brown, *J. Org. Chem.*, **31**, 178 (1966).
(5) E. Fischer, *Justus Liebigs Ann. Chem.*, **215**, 253 (1882).

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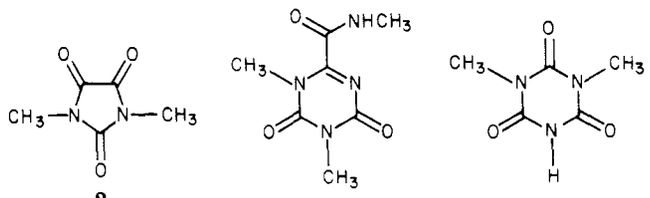
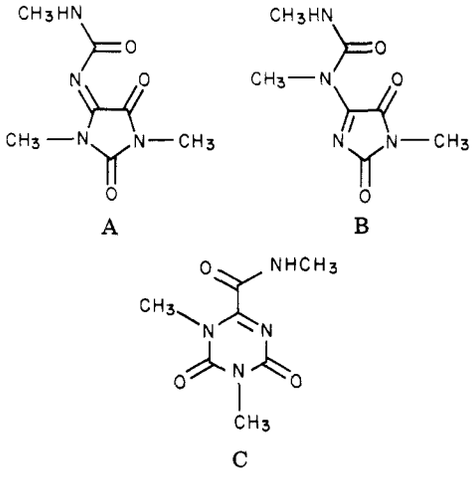


2 3 4

which fragments by loss of 28 mass units. The ^1H NMR spectrum shows three three-proton signals of which two appear as singlets (3.42 and 3.78 ppm) and one appears as a doublet (2.98 ppm). A broad one-proton resonance (7.80 ppm), attributed to an acidic proton attached to nitrogen, is also present. Addition of D_2O to the ^1H NMR solution causes the disappearance of the resonance at 7.8 ppm and the collapse of the doublet (2.98 ppm) to a singlet. This result is consistent with the presence of an *N*-methylamide moiety within the molecule.[6] The ^{13}C NMR spectrum reveals the presence of seven nonequivalent carbon resonances. The mass spectral and NMR spectral data, as well as elemental analysis, demonstrate that the molecule has the formula $C_7H_{10}N_4O_3$. The infrared spectrum shows the presence of a carbonyl absorption (1670 cm^{-1}) and a carbon–nitrogen double bond (1595 cm^{-1}). The presence of three carbonyl groups in the molecule is demonstrated by resonances in the ^{13}C NMR spectrum at 154.5, 156.1, and 159.7 ppm.[7] On the basis of this information, three possible structures (A, B, and C) are considered.



A B C

Compound A was prepared by treatment of acetone cyanohydrin with methyl isocyanate and shown by comparative mass spectral, ^1H NMR, and IR analysis not to be the caffeine ozonation product.[8] Examination of the analytical data on compound **3** did not permit us to distinguish this compound as having either structure B or C, although B was considered unlikely since the only known examples of imidazolidines exist as trimers. Finally, X-ray crystallography was employed to establish the structure of compound **3** as a previously unreported compound shown as C.[9]

The third-ranking compound (**4**) was isolated by column chromatography (silica gel). The mass spectrum gives an apparent molecular ion (*m/e* 157). The structure of 1,3-dimethyl-5-azabarbituric acid (**4**) is proposed on the basis of spectroscopic data, and an authentic sample of **4** was prepared by allowing 2 equiv of methyl isocyanate to react with potassium cyanate.[10] This synthetic sample was found to be identical (mass spectrum, ^1H NMR, IR, melting point) with the caffeine ozonation product.

(6) R. M. Silverstein, G. C. Bassler, and T. C. Morrill, "Spectrometric Identification of Organic Compounds", Wiley, New York, 1974, p 178.
(7) G. C. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists", Wiley-Interscience, New York, 1972, pp 120–5.
(8) T. L. Patton, *J. Org. Chem.*, **32**, 383 (1967).
(9) T. C. Haltiwanger, K. J. Kolonko, R. H. Shapiro, and R. E. Sievers, *Acta Crystallogr.*, in press.

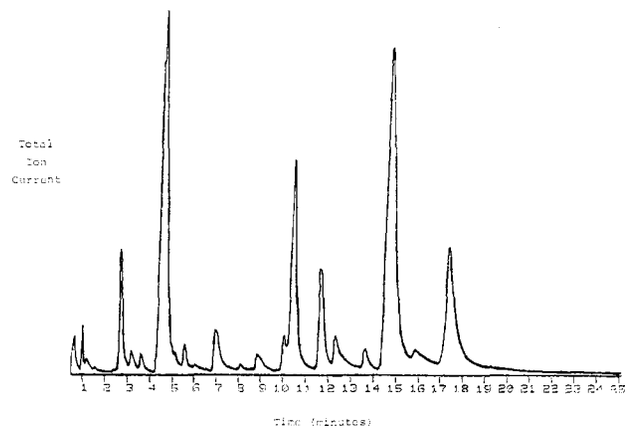
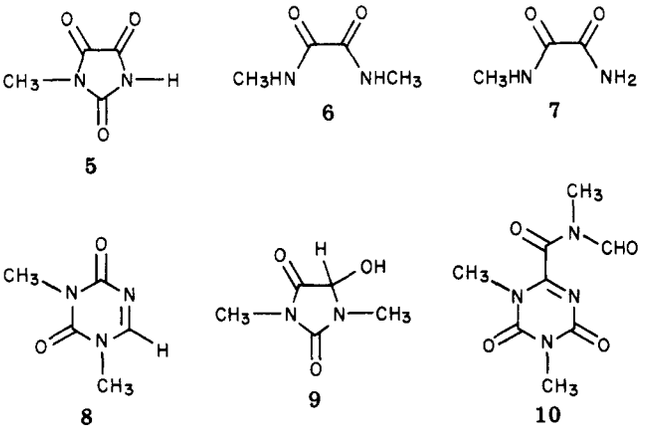


Figure 1. Gas chromatogram of caffeine ozonation products: electron impact mass spectrometry with total ion detection.

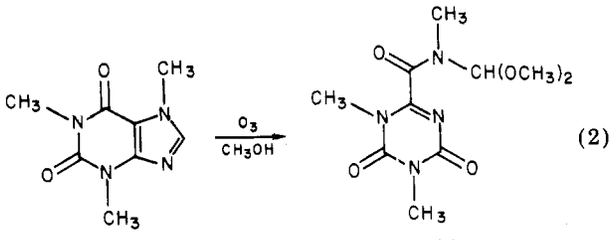
Chart I



5 6 7 8 9 10

The identification of minor reaction products **5**–**10** (Chart I) was based on GC/MS analysis since their yield was very low and their isolation in pure form was not effected. Structural assignments for compounds **5**, **6**, and **8** are substantiated by comparative experiments with authentic samples.

When caffeine (**1**) is allowed to react with ozone in absolute methanol, a new compound (**11**) is produced as the major product (eq 2). Compounds **3** and **10** were not detected in the product mixture. The ^1H NMR spectrum of **11** shows a resonance at 3.26 ppm which indicates the presence of a formyl dimethyl acetal function within the molecule. When **11** was allowed to react with dilute acid, 1,3-dimethyloxonic methylamide (**3**) was produced. This information supports the structural assignment proposed for **10** and suggests that it is a precursor for 1,3-di-



1 11 (2)

(10) P. A. Argabright, B. L. Phillips, and C. H. DePuy, *J. Org. Chem.*, **35**, 2253 (1970).

Table II. Eight Principal Peaks in the Mass Spectra of Caffeine Ozonation Products[a]

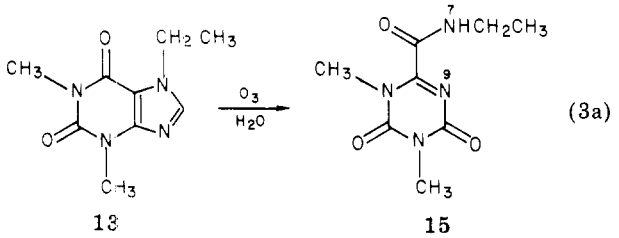
MW	t_r, min	m/e (relative intensity) 1	2	3	4	5	6	7	8
75	1.0	44 (100)	31 (31)	46 (26)	75 (20)	45 (20)	29 (15)		
59	1.2	54 (100)	30 (74)	31 (72)	29 (54)	28 (50)	32 (44)		
117	2.8	58 (100)	89 (20)	28 (13)	59 (9)	15 (9)	117 (6)	29 (6)	
116	3.2	58 (100)	30 (44)	116 (33)	44 (32)	59 (29)	88 (8)		
132	3.6	58 (100)	30 (80)	28 (31)	59 (30)	45 (28)	57 (26)	44 (30)	132 (20)
142	4.5	142 (100)	58 (80)	56 (60)	57 (48)	70 (19)	114 (10)	84 (5)	
102	5.6	58 (100)	31 (65)	32 (56)	30 (45)	44 (43)	59 (30)	102 (10)	
140	7.0	42 (100)	140 (84)	55 (64)	83 (27)	28 (27)	82 (23)		
143	8.9	58 (100)	143 (82)	28 (21)	29 (12)	44 (9)	45 (9)	42 (9)	
144	10.1	59 (100)	58 (87)	42 (70)	116 (51)	144 (23)	88 (22)	56 (18)	127 (7)
157	10.5	157 (100)	58 (74)	56 (51)	57 (31)	70 (22)	44 (11)	100 (9)	129 (8)
141	11.7	141 (100)	42 (98)	39 (37)	56 (30)	84 (30)	41 (18)	29 (12)	83 (12)
128	12.3	56 (100)	128 (70)	57 (61)	100 (41)	43 (34)	70 (24)	44 (22)	42 (16)
210	13.7	42 (100)	182 (41)	56 (34)	69 (20)	167 (11)	97 (7)	210 (5)	142 (5)
198	14.9	56 (100)	83 (80)	44 (44)	58 (42)	170 (38)	141 (32)	198 (31)	113 (7)
254	15.9	42 (100)	127 (61)	56 (33)	169 (24)	70 (24)	28 (22)	128 (19)	254 (15)
226	17.4	83 (100)	45 (78)	30 (37)	28 (28)	156 (24)	184 (17)	141 (17)	226 (6)

[a] Isotope peaks are ignored.

methyloxonic methylamide (3) which is trapped by methanol upon caffeine ozonation.

The origin of specific atoms of the caffeine ozonation products dimethylparabanic (2) and 1,3-dimethyloxonic methylamide (3) was determined by using ethyl and trideuteriomethyl labels. 1-Ethyl-3,7-dimethylxanthine (12) was prepared by treating an alkaline solution of 3,7-dimethylxanthine with ethyl iodide. In a similar manner, 1,3-dimethyl-7-ethylxanthine (13) was prepared from 1,3-dimethylxanthine. The trideuteriomethyl derviatives were prepared by treating the appropriate dimethylxanthine with an alkaline solution of dimethyl-d_6 sulfate.[11]

1,3-Dimethyl-7-ethylxanthine (13) was allowed to react with ozone under the same conditions employed for the ozonation of caffeine (eq 3a). The product mixture was



(3a)

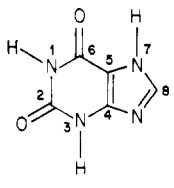
13 15

analyzed by GC/MS techniques, which revealed that products similar to those produced from caffeine (1), but differing by 14 mass units, are produced. A mixture of dimethylparabanic acid (2) and ethylmethylparabanic acid (14) is produced, the former being dominant. A dimethylethyloxonamide (15) is also formed. Finally, the production of methylparabanic acid (5) is accompanied by that of its homologue, ethylparabanic acid (16).

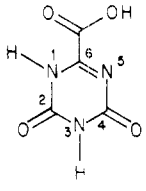
The dimethylethyloxonamide 15 was isolated from the product mixture by preparative column chromatography on silica gel. The ^{1}H NMR spectrum (see Table III) exhibits five resonances: two three-proton singlets (3.40 and 3.78 ppm), a three-proton triplet (1.26 ppm), a broad, unresolved two-proton multiplet (3.50 ppm), and a broad one-proton singlet (8.00 ppm). The signal at 8.00 ppm disappears upon addition of D_2O to the ^{1}H NMR solution and appears to be caused by an exchangeable nitrogen proton. The appearance of two methyl singlets indicates that these methyl groups are attached to nitrogen atoms of the triazine ring since neither resonance is coupled to the nitrogen proton of the carboxamide side chain. The ethyl group must be present in the carboxamide side chain. Structure 15 is consistent with these data and demonstrates that N-7 of compound 13 becomes the nitrogen atom of the carboxamide side chain. Apparently the imino nitrogen of the triazine ring originates from the 9-position of the xanthine molecule.

The ozonation of 1-ethyl-3,7-dimethylxanthine (12) generates a product profile similar to that formed during caffeine (1) ozonation (eq 3b). A different dimethylethyloxonamide (17) is formed along with a mixture of dimethylparabanic acid (2) and ethylmethylparabanic acid (14) in which the latter is dominant. In addition, 5, 6, 16, ethylmethyl-5-hydroxyhydantoin (18), and 1-ethyl-3-

Table III. ^{1}H NMR Data on Xanthine and Oxonic Acid Derivatives



xanthine

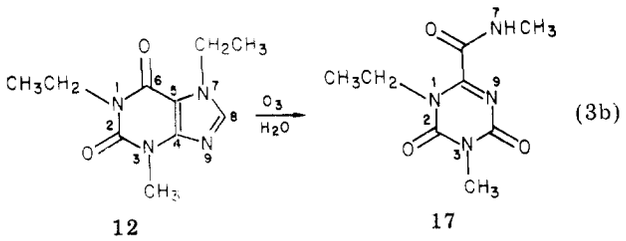


oxonic acid

compd	δ (multiplicity[a]) N_1-alkyl	N_3-alkyl	N_7-alkyl
1,3,7-trimethylxanthine (1)	3.45 (s)	3.55 (s)	4.00 (s)
1,3-dimethyloxonic methylamide (3)	3.78 (s)	3.42 (s)	2.98 (d)[b]
1-ethyl-3,7-dimethylxanthine (12)	4.10 (q)[c]	3.58 (s)	4.00 (s)
1,3-dimethyl-7-ethylxanthine (13)	3.40 (s)	3.68 (s)	4.40 (q)[c]
1-ethyl-3-methyloxonic methylamide (17)	4.44 (q)[c]	3.44 (s)	3.00 (m)[c]
1,3-dimethyloxonic ethylamide (15)	3.78 (s)	3.40 (s)	3.50 (m)[c]
1,3-dimethylurea			3.00 (d)[b]

[a] Multiplicity symbols: s = singlet, d = doublet, q = quartet, m = multiplet. [b] The methyl signal is split into a doublet by the proton attached to nitrogen. [c] Only the methylene proton of the ethyl group is shown. The methyl protons appear as a triplet centered at 1.3-1.5 ppm in each case.

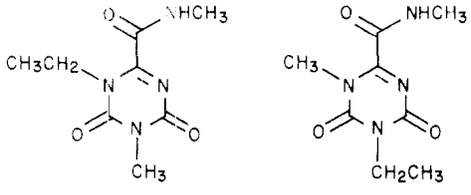
(11) E. V. Dehmlow, *Angew. Chem., Int. Ed. Engl.*, 13, 170 (1974).



12 17 (3b)

methyl-5-azabarbituric acid (**19**) are formed.

The dimethylethyloxonamide **17** was isolated by preparative column chromatography on silica gel. The ^1H NMR (Table III) gives five resonances: a three-proton triplet (1.40 ppm), a three-proton doublet (3.00 ppm), a three-proton singlet (3.44 ppm), a two-proton quartet (4.4 ppm), and a broad one-proton singlet (7.76 ppm). The latter signal collapses upon addition of D_2O. Double-resonance experiments show that the doublet (3.00 ppm) is coupled to this broad singlet (7.76 ppm). These results demonstrate that an *N*-methylcarboxamide function is present and that the ethyl group must be positioned on one of the nitrogen atoms of the triazine ring system. Of the two possible dimethylethyloxonamide structures **17** and **20**, structure **17** is more consistent with the ^1H NMR



17 20

chemical shift data (see Table III). The methyl resonance at 3.44 ppm corresponds with that of a methyl group attached to an imide nitrogen as it is in caffeine. Therefore, structure **17** is proposed.

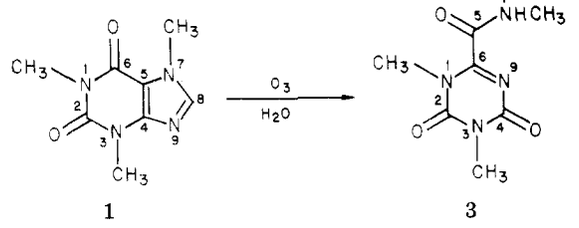
The formation of **17** demonstrates that the 1-, 2-, and 3-positions of 1-ethyl-3,7-dimethylxanthine (**12**) are incorporated in the triazine ring. A probable origin for the remaining atoms is shown in Scheme I. This designation assumes that minimal bond cleavage and recombination occur during product formation. In this scheme the 4-position of caffeine (**1**) becomes the remaining carbonyl of the triazine and the 5-position becomes the carbonyl carbon of the carboxamide side chain. Apparently the 6-position becomes the imino carbon atom.

The results of these labeling experiments can be rationalized in terms of the mechanism shown in Scheme II. Ozonation of caffeine (**1**) occurs to yield the nine-membered ring structure D. The carbon–nitrogen double bond of D is hydrolyzed to yield E. The 9-position (caffeine numbering) of E undergoes nucleophilic addition to the 6-position with subsequent loss of water to form the triazine ring system of F. The formyl group in F is oxidized to an acid (G) which upon decarboxylation yields product **3**.

The appearance of dimethyl- and ethylmethylparabanic acid in the ozonation products of **12** and **13** indicates that dimethylparabanic acid is formed by two or more mechanistic pathways. Apparently dimethylparabanic acid (**2**) is formed in each case from combination of the N-1 and N-3 positions and the N-1 and N-7 positions of caffeine (**1**). It is also possible that some dimethylparabanic acid (**2**) is formed from the N-3 and N-7 combination.

The relative amount of dimethylparabanic acid (**2**) formed from each possible combination was obtained by ozonation of caffeine analogues containing trideuteriomethyl labels (compounds **21**, **23**, and **24**). The aqueous

Scheme I



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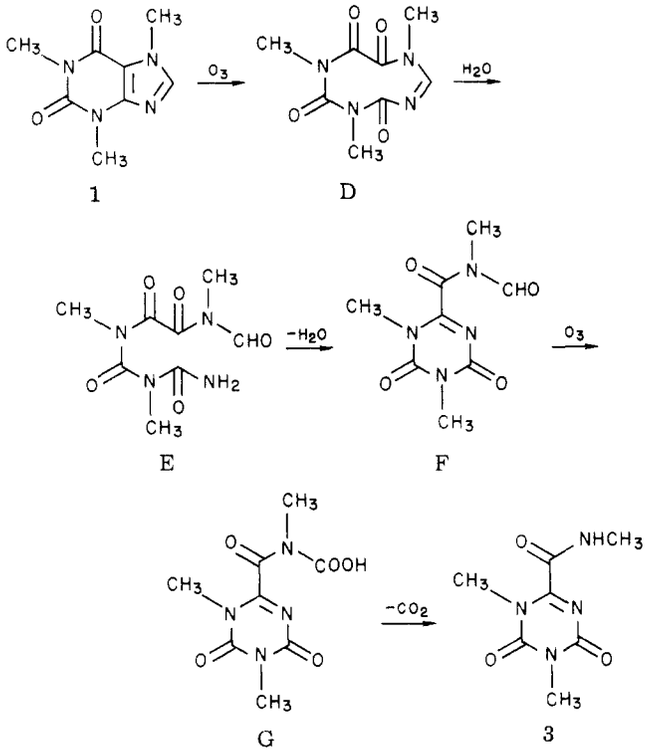
Scheme II



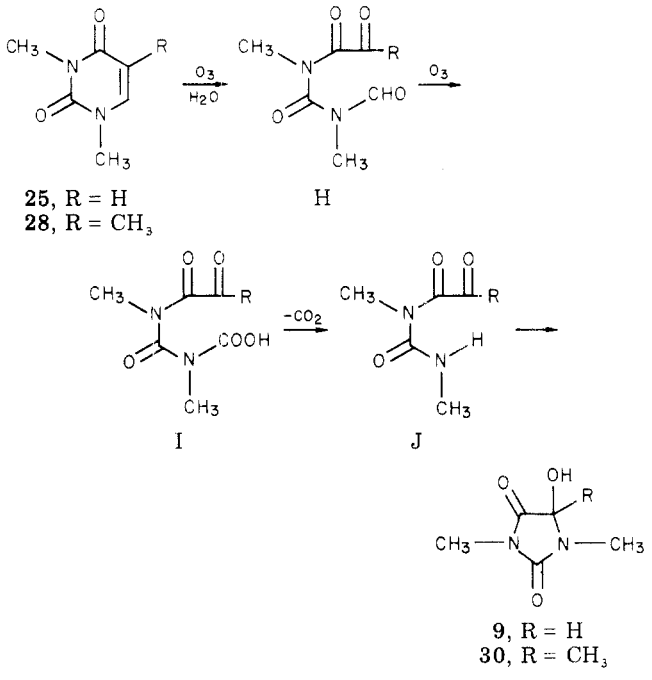
1 D E F G 3

ozonation of each of these labeled analogues (see Table IV) produces dimethylparabanic acid (**2**) which contains both unlabeled and labeled (d_3) methyl groups. The integration of the molecular ion intensity of the unlabeled (m/e 142) vs. labeled (m/e 145) material indicated the relative amount of unlabeled and labeled dimethylparabanic acid (**2**) contained in each product mixture. Since unlabeled dimethylparabanic acid (**2**) can be formed by only one combination of methyl groups in each experiment, the relative percentage formed is the relative contribution formed from the pathway. Ozonation of caffeine analogue **21** labeled at the N-1 position yields 2% unlabeled dimethylparabanic acid (**2**) and 98% labeled (d_3) dimethylparabanic acid (**22**). This example indicates that 2% of the dimethylparabanic acid (**2**) is formed from combination of positions N-3 and N-7. The remaining examples (**23** and **24**) indicate that 12% of the dimethylparabanic acid (**2**) pool is formed from combination of the N-1 and N-7 positions and the remaining 86% is formed from combination of the N-1 and N-3 positions.

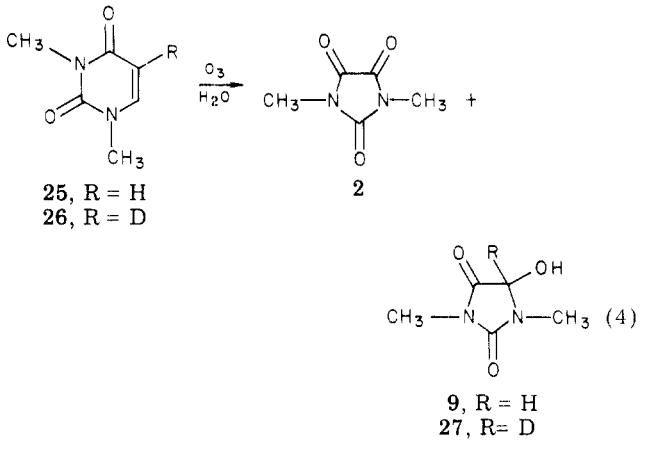
Since most of the dimethylparabanic acid (86%) originates from the pyrimidine ring system of caffeine, the ozonation of the corresponding monocyclic compound 1,3-dimethyluracil (**25**) was investigated to see if similarities exist. 1,3-Dimethyluracil was prepared by allowing uracil to react with an alkaline solution of dimethyl sulfate.[12] The ozonation of 1,3-dimethyluracil (**25**) yields two

(12) D. Davidson and O. Baudisch, *J. Am. Chem. Soc.*, **48**, 2379 (1926).

Scheme III



major products, dimethylparabanic acid (2) and 1,3-dimethyl-5-hydroxyhydantoin (9) (eq 4). Compound 9 was



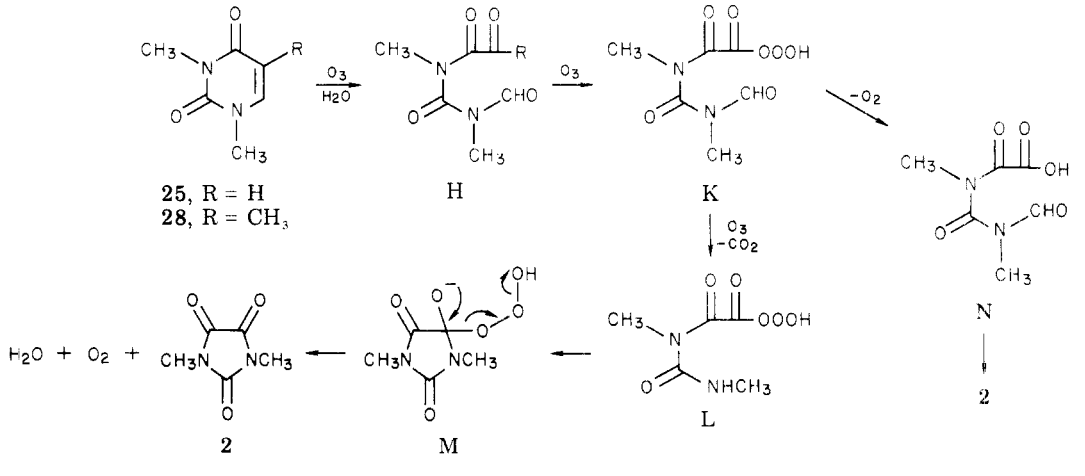
isolated from the product mixture by column chromatography and shown by mass spectrometry to have an apparent molecular weight of 144. The ^1H NMR spectrum shows three resonances: a six-proton singlet (3.02 ppm), a one-proton singlet (5.20 ppm), and a one-proton singlet (5.50 ppm). Addition of D_2O to the ^1H NMR solution causes the singlet at 5.50 ppm to collapse. The infrared spectrum shows an alcohol absorption (3350 cm^{-1}) and a carbonyl absorption (1710 cm^{-1}). When 9 is allowed to react with ozone under the 1,3-dimethyluracil (25) ozonation conditions, starting material is recovered unchanged. If the ozonation is conducted in the presence of $FeSO_4$, 9 is completely converted to dimethylparabanic acid (2).

Table IV. Dimethylparabanic Acid Formation from Deuterium-Labeled Caffeine Analogues

	rel %	
	$M^{+\cdot}$ 142	$M^{+\cdot}$ 145
compd	2	22
21	2	98
23	12	88
24	86	14

The origin of the methine proton at the 5-position of 9 was determined by the ozonation of 1,3-dimethyl-5-deuteriouracil (26). This compound was prepared in good yield (89%) and high isotopic purity (99% d_1) by acid-catalyzed exchange of 1,3-dimethyluracil (25) in deuterium oxide. The ozonation of 26 produces dimethylparabanic acid (2) and 1,3-dimethyl-5-hydroxy-5-deuteriohydantoin (27, 99% d_1). This result demonstrates that the 5-position of 1,3-dimethyluracil (25) is retained in the product and becomes the 5-position of 1,3-dimethyl-5-hydroxyhydantoin (9). These results can be rationalized by the mechanism shown in Scheme III.

Scheme IV

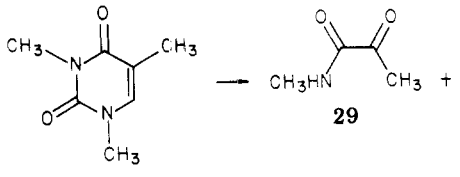


A possible rationale for the formation of dimethylparabanic acid (2) is presented in Scheme IV. 1,3-Dimethyluracil (25) is ozonized to dialdehyde H. In this case, the 5-position (uracil numbering) is oxidized to a hydrotrioxide intermediate K.[13] This intermediate K undergoes further oxidation with concomitant loss of CO_2 to form amide L. Amide L cyclizes to form tetrahedral intermediate M. Intermediate M loses water and singlet oxygen to produce dimethylparabanic acid (2). Alternatively, hydrotrioxide intermediate K could decompose to acid N which then undergoes conversion by a similar scheme to dimethylparabanic acid (2).

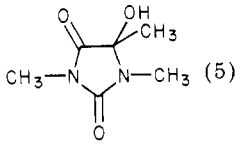
Another possible mechanism for dimethylparabanic acid (2) formation is shown in Scheme V. In this scheme, ozonation of 1,3-dimethyluracil (25) occurs to give ozonide O.[14] Alternatively, an epoxide P might be formed. Either ozonide O or epoxide P undergoes rearrangement to form aldehyde Q. Aldehyde intermediate Q undergoes tautomerization to enol form R which produces dimethylparabanic acid (2) upon ozonation. Precedent for rearrangement of an acyl-substituted epoxide is found in the conversion of chalcone epoxides to α-formyldeoxybenzoins.[15]

The validity of the proposed mechanism shown in Scheme IV is substantiated by the fact that 1,3,5-trimethyluracil (28) does not yield any dimethylparabanic acid (2) upon ozonation. The ozonation of 1,3,5-trimethyluracil (28) proceeds to yield two major products, *N*-methylpyruvamide (29) and 1,3,5-trimethyl-5-hydroxyhydantoin (30) (eq 5). If ozonation of 1,3,5-trimethyluracil (28) proceeds as shown in Scheme V, then an acyl hydantoin (Q) would be formed. Tautomerization of intermediate Q should not be prevented by the presence of a methyl group and thus enol R should be formed. Upon ozonation, intermediate R should produce dimethylparabanic acid (2), a result which is not consistent with experimental fact. However, ozonation of 1,3,5-trimethyluracil (28) according to Scheme IV would produce intermediate H. In this case a methyl group attached to the 5-position would prevent ozonation of the acyl function (intermediate H) to a hydrotrioxide (M) or an acid (N). Dimethylparabanic acid (2) formation according to this mechanism would be prevented.

The proposal that intermediate H is formed upon ozonation of 1,3,5-trimethyluracil (28) is supported by the production of 1,3,5-trimethyl-5-hydroxyhydantoin (30) during the reaction. Apparently 30 is formed by oxidation of the formyl moiety of intermediate H to an acid which upon decarboxylation yields intermediate J (see Scheme III). Intermediate J cyclizes to yield 1,3,5-trimethyl-5-hydroxyhydantoin (30).



28 29



30 (5)

Scheme V

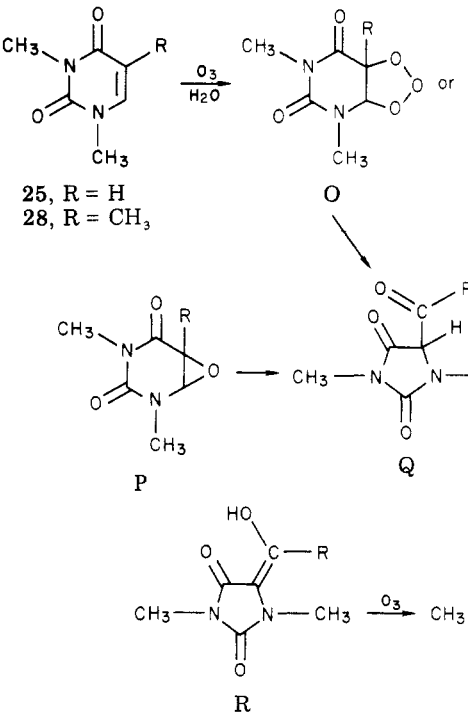
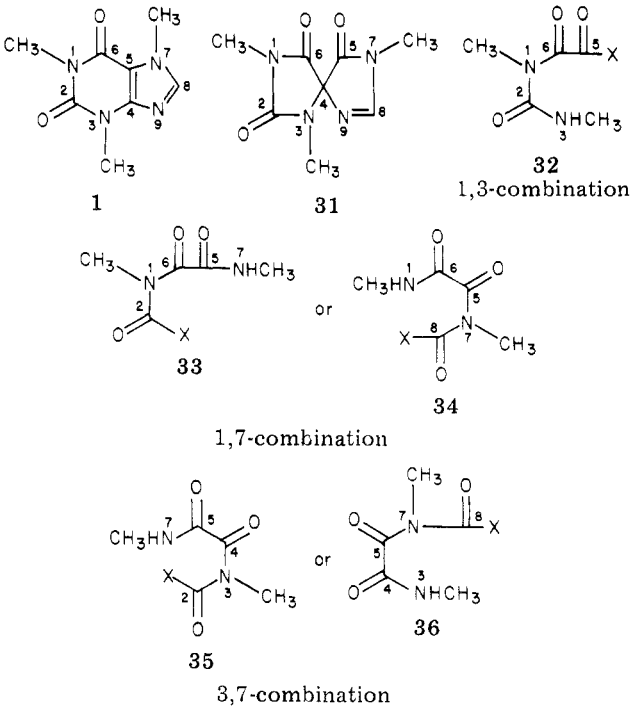


Chart II



Conclusion

The ozonation of caffeine (1) yields several major products and many minor products. These results demonstrate that the ozonation reaction of xanthine and pyrimidine derivatives occurs by diverse mechanistic paths. 1,3-Dimethyloxonic methylamide (3) is formed upon caffeine (1) ozonation by one distinct mechanistic pathway. Dimethylparabanic acid (2), however, is formed upon caffeine (1) ozonation by at least three distinct mechanistic pathways. If the ozonation of caffeine proceeds by the type

(13) F. E. Stary, D. E. Emge, and R. W. Murray, *J. Am. Chem. Soc.*, 98, 1880 (1976).
(14) L. I. Smith, F. L. Greenwood, and O. Hudrlik, "Organic Syntheses", Collect Vol. III, Wiley, New York, 1955, p 673.
(15) S. N. Seshagiri, R. K. Gupta, and M. Krishmurit, *Indian J. Chem.*, 14B, 1011, (1976).

of mechanism shown in Scheme V for 1,3-dimethyluracil (**25**), then the intermediate **31** shown in Chart II would be formed. Blitz proposed that similar spiro compounds are oxidative reaction products of substituted uric acids.[16] Ozone-induced decomposition of intermediate **31** to dimethylparabanic acid (**2**) would explain only one mechanism for dimethylparabanic acid (**2**) formation. All three mechanistic pathways can be explained if intermediates of the type shown in Chart II (**32–36**) are formed upon caffeine (**1**) ozonation. Intermediates of this type might result from ozone-induced decomposition of intermediates D or E shown in Scheme II. In Chart II, X represents a suitable leaving group: a hydroxyl, hydrotrioxide, or peroxide linkage are possible leaving groups. The results of this study indicate that Scheme IV is the more likely mechanism for dimethylparabanic acid (**2**) formation. However, neither Scheme IV nor Scheme V, nor a combination of both, can be excluded by out data.

Experimental Section

Organic-free distilled water was obtained by distillation from a $KMnO_4$–KOH (50:50) solution. Methyl isocyanate, 5-azauracil, dimethyl sulfate, oxalyl chloride, ethylurea, 1,3-dimethylurea, ethyl iodide, and Diazald were obtained from Aldrich Chemical Co., Inc. Caffeine and methylurea were obtained from Eastman Kodak Co. Theobromine, theophylline, thymine, and uracil were obtained from Sigma Chemical Co. Potassium cyanate was obtained from Matheson Coleman and Bell. Dimethyl-d_6 sulfate was obtained from Merck Sharp & Dohme Canada Ltd. 1,3-Dimethyl-4-(methylcarbamoylimino)-1,3-imidazoline-2,5-dione was prepared by the procedure of Patton.[8] Chlorine gas was obtained from the Matheson Gas Co. Silica gel (Woelm) (0.031–0.063 mm) for column chromatography was obtained from ICN Pharmaceuticals Inc. Precoated thin-layer chromatography plates (silica gel, thickness 0.25 mm) were obtained from Van Waters and Rogers Scientific.

Liquid chromatography was conducted on a 30 × 1000 mm Altec glass column packed with silica gel (Woelm) and equipped with a 15 × 150 mm precolumn (silica gel). Solvents were delivered at 100 psi by a fluid metering pump (FMI, Model RSPY). Typcial flow rates range from 10 to 19 mL/min. Gas chromatography–mass spectrometry (GC/MS) was conducted on a Hewlett-Packard 5982 A GC/MS computer system. A membrane separator was employed to interface the gas chromatograph to the mass spectrometer. Proton magnetic resonance (^{1}H NMR) spectra were obtained on a Varian EM-390 90-MHz spectrometer. Carbon magnetic resonance (^{13}C NMR) spectra were obtained on a JEOL PFT-100 spectrometer equipped with a Nicolet Model 1080 data system. Infrared spectra were obtained on a Perkin-Elmer 337 grating infrared spectrometer. Melting points were obtained on a Thomas Hoover capillary melting point apparatus and are corrected. Gas chromatography was conducted on a Hewlett-Packard Model 5830 gas chromatograph equipped with glass columns and a flame ionization detector. Gas chromatography conditions are as follows.

Conditions A: column, Aue pack[17] (2 mm × 10 ft), 100–120 mesh; temperature 1, 100 °C; time 1, 0 min; rate, 8 °C/min; temperature 2, 240 °C; time 2, 10 min; injection temperature, 250 °C; flow (N_2), 30 mL/min; mass scan, 10–300 mass units.

Conditions B: column, Aue pack (2 mm × 10 ft), 100–120 mesh; temperature 1, 100 °C; time 1, 0 min; rate, 10 °C/min; temperature 2, 240 °C; time 2, 30 min; injection temperature, 240 °C; FID temperature, 250 °C; chart speed, 0.5 in./min.

Conditions C: column, DC-710 (5 %, 2 mm × 10 ft), on Chromosorb Q, 80–100 mesh; temperature 1, 150 °C; time 1, 4 min; rate, 16 °C/min; temperature 2, 220 °C; time 2, 20 min; injection temperature, 250 °C; flow (N_2), 30 mL/min; mass scan, 10–300 mass units.

Conditions D: column, Aue pack (2 mm × 6 ft), 100–120 mesh; conditions B were used except the slope sensitivity was 1.00.

It was determined that the Aue pack columns (conditions A, B, and D) were best suited for chromatography of xanthine derivatives and their ozonation products.

Caffeine in the Upper Thompson Sanitation District.[18] A waste water sample was obtained from the Upper Thompson Sanitation District on June 15, 1976, at 12:30 p.m. The sample was obtained prior to entry into the ozonation basin, the final step in the treatment process. The sample was packed in ice and transferred to the University of Colorado, Boulder, and stored at 4 °C until analysis. A 1-gal aliquot was allowed to warm to room temperature and a pH measurement of 7.2 was obtained. A 800-mL aliquot was filtered through sintered glass and transferred to a separatory funnel. The pH of this solution was adjusted to greater than pH 10 by addition of 50% NaOH. The resulting solution was extracted three times with 133 mL of $CHCl_3$, and the organic layers were combined and passed through a short column of Na_2SO_4 (20 g). The solution was placed in a Kuderna-Danish vessel and concentrated. The concentrated sample was transferred to a vial and concentrated to several drops under a gentle stream of N_2. The resulting sample was analyzed by gas chromatography–mass spectrometry. The GC conditions were as follows: column, 3% OV-1 on 80–100 mesh Chromosorb W, 6 ft × 2 mm; injection temperature, 200 °C; flow (N_2), 30 mL/min; temperature 1, 75 °C; time 1, 2 min; rate, 4 °C/min; temperature 2, 250 °C. Under these conditions, the GC/MS experiment provided spectra from which caffeine was the only identifiable component. Excessive column bleed precluded the identification of other components in the chromatogram. The caffeine gave a retention time of 22.0 min and a mass spectrum (70 eV) with m/e (relative intensity) 80 (15), 193 (27), 109 (45), 82 (51), 67 (60), and 194, M^+ (100). This structural assignment was verified by the coinjection of an authentic sample of caffeine with the original sample. Caffeine (authentic sample): mass spectrum (70 eV) m/e (relative intensity) 82 (16), 193 (16), 55 (27), 67 (27), 109 (44), 194, M^+ (100).

Ozonation of Caffeine (1). Caffeine (0.5 g, 2.6 mmol) was dissolved in pure distilled water (750 mL) and placed in a 1-L, three-necked flask equipped with a magnetic stirrer, a gas dispersion tube, and a bubble flow meter. The solution was allowed to react for 90 min with an ozone–oxygen stream (600 mL/min, 17 mg of O_3/min). The resulting solution was sparged with nitrogen (600 mL/min) for 20 min, transferred to a round-bottomed flask, and concentrated by rotoevaporation. The residue was dissolved in 50 mL of CH_3OH–CH_2Cl_2 (50:50) and dried over anhydrous Na_2SO_4. This mixture was filtered and concentrated by rotoevaporation. The residue was transferred to a vial and the volume adjusted to 1.0 mL by addition of CH_3OH. This sample was analyzed by gas chromatography–mass spectrometry to yield the data given below. Gas chromatography was conducted to determine the relative product distribution (results are not corrected for variance in detector response). Coinjection experiments were conducted according to conditions D. The data are presented in the following form. Compound: retention time for conditions A (relative percent for conditions B); mass spectrum (70 eV) m/e (relative intensity). **1,4-Dimethyloxalamide**: 3.2 min (0.6%); MS 88 (8), 59 (29), 44 (32), 116, M^+ (33), 30 (44), 59 (100). Coinjection, ozonation product, t_r 3.80 min (coinjection with authentic sample, t_r 3.87 min). **Dimethylparabanic acid**: 4.5 min (23.0%), MS 48 (5), 114 (10), 70 (19), 57 (48), 56 (60), 58 (80), 142, M^+ (100). Coinjection, ozonation product, t_r 4.98 min (coinjection with authentic sample, t_r 5.09 min). **1-Methyloxalamide**: 5.6 min (1.6%); MS 45 (10), 102, M^+ (10), 59 (30), 44 (34), 30 (45), 32 (56), 31 (65), 58 (100). **1,3-Dimethyl-5-hydroxyhydantoin**: 10.0 min (2.7%); MS 127 (7), 56 (18), 88 (22), 144, M^+ (23), 116 (51), 42 (70), 58 (87), 59 (100). Coinjection, ozonation product, t_r 9.81 min (coinjection with authentic sample, t_r 10.01 min). **1,3-Dimethyl-5-azabarbituric acid**: 10.5 min (8.7%); MS 129 (8), 100 (9), 44 (19), 70 (22), 57 (31), 56 (51), 58 (74), 157, M^+ (100). Coinjection, ozonation product, t_r 10.07 min (coinjection with authentic sample, t_r 10.13 min). **1,3-Di-**

(16) (a) H. Biltz and M. Heyn, *Justus Liebigs Ann. Chem.*, **413**, 7–60 (1916); (b) H. Biltz and M. Bergius, *ibid.*, **414**, 54–7 (1917).

(17) W. A. Aue, C. R. Hastings, and S. Kapila, *J. Chromatogr.*, **77**, 299 (1973).

(18) The Upper Thompson Sanitation District is located in Estes Park, Colo.

methyl-5-azauracil: 11.7 min (3.4%); MS 83 (12), 29 (12), 41 (18), 84 (30), 56 (30), 28 (37), 42 (98), 141, M^+ (100). Coinjection, ozonation product, t_r 10.93 min (coinjection with authentic sample, t_r 10.94 min). **Methylparabanic acid**: 12.3 min (5.8%); MS 42 (16), 44 (22), 70 (24), 43 (34), 100 (41), 57 (61), 128, M^+ (70), 56 (100). Coinjection, ozonation product, t_r 11.53 min (coinjection with authentic sample, t_r 11.57 min). **1,3-Dimethyloxonic methylamide**: 14.9 min (26.4%); MS 113 (7), 198, M^+ (31), 141 (32), 170 (38), 58 (42), 44 (44), 83 (80), 56 (100).

Determination of Ozone–Caffeine Stoichiometry. A 1-L, three-necked flask (reaction vessel) containing pure water (750 mL) was placed in line with a 2-L, three-necked flask containing 1.5 L of 2% KI solution. Ozone–oxygen (600 mL/min) was passed through the system for 1.5 h. The 1.5-L solution of KI–I_2 was transferred to a 2-L volumetric flask and diluted to volume with water. Three 100-mL aliquots were removed and titrated with standard $Na_2S_2O_3$ solution (0.1031 N) to give the following results: titer, 31.84, 31.94, and 31.94 mL. The amount of ozone produced within a 1.5-h period was determined to be 1.58 g (17.7 mg of O_3/min). A second experiment was conducted under the above conditions with caffeine (0.5000 g, 2.57 mmol) present in the reaction vessel. The amount of unreacted ozone which passed into the KI trap in a 1.5-h period was determined to be 1.07 g of O_3 (KI titer, 21.52, 21.60, and 21.56 mL; average 21.56 mL). The amount of O_3 which reacted with caffeine was found to be 0.52 g or 10.2 mmol of ozone. The number of moles of ozone reacted per mole of caffeine reacted was 4.2 mol of ozone per mol of caffeine.

Isolation of Dimethylparabanic Acid (2). Caffeine (1.0 g, 5.2 mmol) in water (750 mL) was ozonized as described above (ozonation time, 3 h). The solution was sparged with N_2 (600 mL/min) and extracted three times with $CHCl_3$ (200 mL). The $CHCl_3$ layers were combined, dried over Na_2SO_4, and filtered. The resulting filtrate was concentrated by rotoevaporation. The remaining residue was analyzed by gas chromatography (conditions A) and found to contain one component (retention time, 6.9 min). This residue crystallized upon cooling and was vacuum-dried. The crystalline material was recrystallized from 95% ethanol, collected by suction filtration, and vacuum-dried to yield 0.2 g (27%) of dimethylparabanic acid (**2**), mp 153 °C (lit.[19] mp 155 °C).

Isolation of 1,3-Dimethyloxonic Methylamide (3). A sample of caffeine (5.0 g, 25.8 mmol) in water (750 mL) was allowed to react with an ozone–oxygen stream (600 mL/min, 17 mg of O_3/min) for 50 min. The mixture was sparged with N_2 (600 mL/min) for 20 min and concentrated by rotoevaporation. This residue was transferred to a vial and the volume adjusted to 10 mL with 50:50 CH_2Cl_2–CH_3OH. Thin-layer chromatography (TLC) of this sample on silica gel with THF–EtOAc (50:50) showed three major products: **1,3-Dimethylparabanic acid (2)** (R_f 0.63), **1,3-dimethyloxonic methylamide (3)** (R_f 0.40), and **caffeine (1)** (R_f 0.20). This sample was subjected to column chromatography through silica gel with THF–EtOAc (50:50). Fractions were collected at 10-mL intervals and analyzed by TLC. The fractions containing 1,3-dimethyloxonic methylamide (**3**) were combined and concentrated. This concentrated sample was again chromatographed through silica gel to yield fractions containing pure product. These fractions were combined and concentrated by rotoevaporation to yield an oil which crystallized upon scratching. This material was vacuum-dried to yield 65 mg of 1,3-dimethyloxonic methylamide (**3**), mp 178–179 °C. Anal. Calcd for $C_7H_{10}N_4O_3$: C, 42.42; H, 5.09; N, 28.27. Found: C, 42.78; H, 5.00; N, 28.03.

Isolation of 1,3-Dimethyl-5-azabarbituric Acid (4). A sample of caffeine (20.0 g, 103 mmol) in water (1500 mL) was allowed to react with an ozone–oxygen stream (600 mL/min, 17 mg of O_3/min) for 20 min and extracted four times with CH_2Cl_2 (250 mL) to remove dimethylparabanic acid from the aqueous phase. The aqueous phase was concentrated to an oil, which was dissolved in EtOH–CH_2Cl_2 (200 mL, 50:50) and passed through a short column of anhydrous Na_2SO_4. This dry organic phase was concentrated to 30 mL. A 15-mL aliquot of this sample was chromatographed through silica gel with $CHCl_3$–THF–EtOAc (60:20:20). Chromatographic fractions were collected at 20-mL intervals and allowed to evaporate to dryness. The crystalline materials which were isolated in fractions 21–40 were combined and recrystallized (EtOH) to yield 100 mg of 1,3-dimethyl-5-azabarbituric acid, mp 220–221 °C. Physical properties were identical with those of synthetic material (see below).

Preparation of 1,3-Dimethyl-5-azabarbituric Acid (4). A mixture of potassium isocyanate (2.0 g, 24.6 mmol) and methyl isocyanate (5.7 g 100 mmol) in DMF (100 mL) was allowed to react for 24 h at 75 °C. The DMF was removed by evaporation to yield a solid residue. The residue was slurried in water (50 mL) and filtered to remove 1,3,5-trimethyl-5-azabarbituric acid. The filtrate was acidified with concentrated HCl and filtered to yield 2.2 g of crude 1,3-dimethyl-5-azabarbituric acid (57%), mp 221–222 °C (lit.[10] mp 222–223 °C).

Preparation of *N*-Methylparabanic Acid (5). A mixture of methylurea (4.5 g, 60.8 mmol) and oxalyl chloride (7.5 g, 59.1 mmol) in Et_2O was refluxed for 2 h. The Et_2O was removed by rotoevaporation to yield a crystalline solid. The solid was recrystallized to yield 5.3 g of *N*-methylparabanic acid (70%), mp 155–157 °C (lit.[4d] mp 148 °C).

Preparation of 1,3-Dimethyl-5-azauracil (8). 5-Azauracil (0.2 g, 1.77 mmol) was dissolved in a solution of NaOH (0.2 g, 5.0 mmol) in water (5 mL). Dimethyl sulfate (0.5 mL, 5.3 mmol) was added and the solution brought to a gentle boil. The solution was allowed to cool to room temperature and extracted three times with CH_2Cl_2 (5 mL). The CH_2Cl_2 extracts were combined and passed through a short column of anhydrous Na_2SO_4. The CH_2Cl_2 was removed by evaporation to yield 0.1 g of 1,3-dimethyl-5-azauracil (40%). Analytically pure material was obtained upon recrystallization from EtOH, mp 155–157 °C (lit.[20] mp 164 °C).

Ozonation of Caffeine (1) in Methanol. Caffeine (0.1 g, 5.2 mmol) was slurried in absolute methanol (100 mL) and allowed to react with an ozone–oxygen stream (600 mL/min, 17 mg of O_3/min) for 3 h. The solution was sparged with N_2 (600 mL/min, 20 min) and concentrated by evaporation. The solution was analyzed by GC and allowed to stand in a vial at room temperature. After several months, a large single crystal formed and was removed. The crystal was vacuum-dried to yield 0.1 g of 1,3-dimethyloxonic *N*-methyl-*N*-(dimethoxymethyl)amide (**11**), mp 137–139 °C.

Preparation of 1-Ethyl-3,7-dimethylxanthine (12). Theobromine (10.0 g, 55.6 mmol) was slurried in EtOH (50 mL), and a solution of KOH (5.0 g, 89.2 mmol) in EtOH (50 mL) was added. When the solution was cooled to 40 °C, a precipitate formed. A minimum amount of water (30 mL) was added to dissolve the precipitate. A solution of ethyl iodide (15.8 g, 101 mmol) in EtOH (20 mL) was added and the mixture refluxed for 6 h. The solution was concentrated to 50 mL by evaporation and 50 mL of water was added. The mixture was extracted three times with CH_2Cl_2 (100 mL). The CH_2Cl_2 layers were combined, dried over anhydrous Na_2SO_4, filtered, and evaporated. The resulting solid was sublimed to yield 9.0 g of 1-ethyl-3,7-dimethylxanthine (78%), mp 163–165 °C (lit.[21] mp 164–165 °C).

Preparation of 1,3-Dimethyl-7-ethylxanthine (13). Following the above procedure for preparation of 1-ethyl-3,7-dimethylxanthine, theophylline (10.0 g, 55.6 mmol), KOH (5.0 g, 89.2 mmol) in EtOH (50 mL), and ethyl iodide (15.8 g, 101 mmole yielded 9.5 g of 1,3-dimethyl-7-ethylxanthine (82%), mp 148–150 °C (lit.[22] mp 154 °C).

Ozonation of 1-Ethyl-3,7-dimethylxanthine (12). Following the above procedure for the ozonation of caffeine, a quantity of 1-ethyl-3,7-dimethylxanthine (0.5 g, 2.4 mmol) in water (750 mL) was allowed to react with ozone–oxygen. The product mixture was subjected to gas chromatographic–mass spectral analysis (conditions C). The following table lists the products and their retention times.

(19) M. Menschutkin, *Justus Liebigs Ann. Chem.*, 178, 202 (1875).

(20) H. Biltz and R. Robl, *Ber. Dtsch. Chem. Ges.*, **54**, 2448 (1921).

(21) W. van der Slooten, *Arch. Pharm. (Weinheim, Ger.)*, **235**, 469 (1897).

(22) E. Schmidt and W. Schwabe, *Arch. Pharm. (Weinheim, Ger.)*, **245**, 312 (1907).

compd	t_r, min
N,N'-dimethyloxalamide (6)	1.8
dimethylparabanic acid (2)	2.5
N-methyl-*N'*-ethylparabanic acid (14)	2.8
1-ethyl-3-methyl-5-hydroxyhydantoin	4.6
N-methyl- and *N*-ethylparabanic acid (5, 16)	5.1
1-ethyl-3-methyl-5-azabarbituric acid	5.8
1-ethyl-3-methyloxonic *N*-methylamide (17)	10.9

Ozonation of 1,3-Dimethyl-7-ethylxanthine (13). Following the above procedure for the ozonation of caffeine, a quantity of 1,3-dimethyl-7-ethylxanthine (0.5 g, 2.4 mmol) in water (750 mL) was allowed to react with ozone–oxygen, and the product mixture was subjected to GC/MS analysis (conditions C, −8 °C/min). The following table lists the products and their retention times.

compd	t_r, min
dimethylparabanic acid (2)	5.4
N-methyl-*N'*-ethylparabanic acid (14)	5.6
N-methyl- and *N*-ethylparabanic acid (5, 16)	8.1
1,3-dimethyl-5-azabarbituric acid (4)	8.1
1,3-dimethyl-5-azauracil (8)	9.9
1,3-dimethyloxonic *N*-ethylamide (15)	13.0

Isolation of Ethylmethylparabanic Acid (14). 1-Ethyl-3,7-dimethylxanthine (5.0 g, 24 mmol) in water (750 mL) was allowed to react for 12 h with an ozone–oxygen stream (600 mL/min, 17 mg of O_3/min). The solution was sparged with N_2 (600 mL/min) for 20 min and extracted three times with CH_2Cl_2 (200 mL). The CH_2Cl_2 extracts were combined, dried over anhydrous Na_2SO_4, filtered, and concentrated by evaporation. The resulting oil was subjected to bulb-to-bulb distillation at reduced pressure to yield 1.1 g of ethylmethylparabanic acid (29%), mp 42–44 °C (lit.[23] mp 44 °C). Spectra were identical with those of synthetic material (see below).

Preparation of Ethylparabanic Acid (16). Following the procedure for preparation of methylparabanic acid, ethylurea (10.0 g, 114 mmol) and oxalyl chloride (14.4 g, 114 mmol) yielded 9.9 g of ethylparabanic acid (61%), mp 91–94 °C (lit.[24] mp 127–128 °C), which was used in the following procedure without further purification.

Preparation of Ethylmethylparabanic Acid (14). Ethylparabanic acid (5.0 g, 35 mmol) in CH_2Cl_2 (250 mL) was allowed to react (3.5 h, room temperature) with excess CH_2N_2 (70 mmol). The excess CH_2N_2 was destroyed with HOAc, and the solvent was removed by rotoevaporation to yield an oil. The oil was distilled at reduced pressure to yield 4.2 g of ethylmethylparabanic acid (76%), mp 42–44 °C.

Isolation of 1-Ethyl-3-methyloxonic Methylamide (17). 1-Ethyl-3,7-dimethylxanthine (10.0 g, 48 mmol) in water was allowed to react for 12 h with an ozone–oxygen stream (600 mL/min, 17 mg of O_3/min). The solution was sparged with N_2 (600 mL/min) and extracted three times with CH_2Cl_2 (200 mL). The aqueous phase was concentrated to an oil by rotoevaporation. The oil was dissolved in 100 mL of CH_2Cl_2–CH_3OH (50:50) and passed through a column of anhydrous Na_2SO_4. This solution was concentrated to 20 mL and chromatographed through silica gel with $CHCl_3$–CH_3OH (19:1). Fractions of the effluent (20 mL) were collected and allowed to evaporate. The composition of these fractions was determined by GC analysis. The contents of fractions 45–47 (which contained the desired product) were combined and evaporated, and the residue was recrystallized from EtOH to yield 1.2 g of 1-ethyl-3-methyloxonic methylamide, mp 182–184 °C.

Isolation of 1,3-Dimethyloxonic Ethylamide (15). 1,3-Dimethyl-7-ethylxanthine (7.5 g, 35 mmol) and $FeSO_4$ (5.4 g, 35 mmol) were dissolved in water (500 mL) and allowed to react 4 h with an ozone–oxygen stream (600 mL/min, 17 mg of O_3/min). The solution was sparged with N_2 (600 mL/min, 20 min) and Celite was added. The slurry was filtered to remove an iron precipitate and the filtrate was concentrated by evaporation. The residue was shaken in the presence of several boiling stones and 100 mL of CH_2Cl_2–CH_3OH (50:50). A small amount of Celite was added and the solution was filtered. The filtrate was concentrated to 10 mL and chromatographed through silica gel with $CHCl_3$–CH_3OH (19:1). Solvent fractions were collected (10 mL) and analyzed by GC. Fractions 120–160 were combined and evaporated to yield 271 mg of 1,3-dimethyloxonic ethylamide, mp 145–147 °C.

Preparation of 1-(Trideuteriomethyl)-3,7-dimethylxanthine (21). Theobromine (1.0 g, 5.4 mmol), triethylbenzylammonium chloride (0.4 g, 1.8 mmol), and sodium hydroxide (0.5 g, 12.5 mmol) were partitioned between chloroform (100 mL) and water (100 mL). Dimethyl-d_6 sulfate (1.0 mL, 10.1 mmol) was added and the mixture refluxed for 1 h. The mixture was poured into a separatory funnel, the layers were separated, and the aqueous phase was extracted twice with 100 mL of chloroform. The chloroform extracts were combined, dried over anhydrous Na_2SO_4, filtered, and concentrated by rotoevaporation. The resulting solid was sublimed at reduced pressure [(130 °C (0.1 mm)] to yield 0.9 g of 1-(trideuteriomethyl)-3,7-dimethylxanthine (85%), mp 231 °C

Preparation of 1,3-Dimethyl-7-(trideuteriomethyl)xanthine (24). Following the above general procedure, theophylline (1.0 g, 5.4 mmol), triethylbenzylammonium chloride (0.4 g, 1.8 mmol), sodium hydroxide (0.5 g, 12.5 mmol), and dimethyl-d_6 sulfate (1.0 mL, 10.1 mmol) yielded 0.9 g of 1,3-dimethyl-7-(trideuteriomethyl)xanthine (85%), mp 234 °C.

Preparation of 1,7-Dimethyl-3-(trideuteriomethyl)xanthine (23). Following the above general procedure, 1,7-dimethylxanthine (100 mg, 0.54 mmol), triethylbenzylammonium chloride (0.1 g, 0.4 mmol), sodium hydroxide (0.1 g, 2.5 mmol), and dimethyl-d_6 sulfate (0.2 mL, 2.0 mmol) yielded 71 mg of 1,7-dimethyl-3-(trideuteriomethyl)xanthine (67%), mp 231 °C.

Preparation of 1,3-Dimethyluracil (25). Uracil (20 g, 178 mmol) and sodium hydroxide (17.0 g, 425 mmol) were dissolved in water (100 mL). The solution was cooled in an ice bath, and dimethyl sulfate (53.2 g, 422 mmol) was added. The solution was brought to a boil, cooled to room temperature, and extracted three times with chloroform (100 mL). The chloroform extracts were combined, dried over Na_2SO_4, filtered, and concentrated by rotoevaporation. The resulting solid was vacuum-dried to yield 22.0 g of 1,3-dimethyluracil (88%), mp 122–124 °C (lit.[12] mp 123–124 °C).

Ozonation of 1,3-Dimethyluracil (25). A solution of 1,3-dimethyluracil (10.0 g, 71 mmol) in water (750 mL) was allowed to react with a stream of ozone–oxygen (600 mL/min, 7 h). The reaction mixture was sparged with N_2 (600 mL/min, 20 min) and the water removed by rotoevaporation. The resulting oil was dissolved in CH_2Cl_2 (100 mL) and passed through a short column of anhydrous Na_2SO_4. The CH_2Cl_2 solution was concentrated to a 20-mL volume and subjected to GC analysis (conditions C). The reaction mixture was found to consist of four major products (85% of the product mixture). Retention times and percents are as follows. **Dimethylparabanic acid (2)**: 3.3 min, 32%. **1,3-Dimethyl-5-hydroxyhydantoin (9)**: 6.1 min, 38%. Compound A: 8.1 min, 11%. Compound B: 11.6 min, 4%. The reaction mixture was chromatographed through silica gel with EtOAc–$CHCl_3$ (80:20) and solvent fractions were collected at 20-mL intervals and analyzed by GLC. Compound **2** was found in fractions 27–32 and compound **9** was found in fractions 54–74. Compounds A and B were not obtained in pure form. Their mass spectra are available as supplementary material. The respective fractions were concentrated by rotoevaporation to yield 1.5 g of dimethylparabanic acid (**2**) and 1.0 g of 1,3-dimethyl-5-hydroxyhydantoin (**9**).

Preparation of 1,3-Dimethyl-5-deuteriouracil (26). 1,3-Dimethyluracil (1.0 g, 71 mmol) was dissolved in D_2O (15 g, 0.75 mmol) and 5 drops of $POCl_3$ was added. The reaction mixture was refluxed overnight, allowed to cool, and extracted three times with CH_2Cl_2 (40 mL). The CH_2Cl_2 layers were combined, dried over Na_2SO_4, filtered, and evaporated. Sublimation [(160 °C (0.1 mm)] yielded 0.9 g of 1,3-dimethyl-5-deuteriouracil (90%), mp 118–120 °C.

Ozonation of 1,3-Dimethyl-5-deuteriouracil (26). Following the procedure for the ozonation of 1,3-dimethyluracil, a quantity of 1,3-dimethyl-5-deuteriouracil (0.1 g, 0.71 mmol) in water (5 mL) was allowed to react with ozone–oxygen. The product mixture was analyzed by gas chromotography–mass spectrometry (conditions A), showing the presence of dimethylparabanic acid (**2**)

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and 1,3-dimethyl-5-hydroxyhydantoin-5-*d* (**27**).

Ozonation of 1,3-Dimethyl-5-hydroxyhydantoin (9). Following the procedure for the ozonation of 1,3-dimethyluracil, 1,3-dimethyl-5-hydroxyhydantoin (0.1 g, 0.7 mmol) dissolved in water (5 mL) was allowed to react with ozone–oxygen. Following workup, the reaction mixture was subjected to gas chromatographic analysis. GC analysis revealed that only starting material, 1,3-dimethyl-5-hydroxyhydantoin, was present in the reaction mixture: t_r 11.27 min; coinjection with authentic sample, t_r 11.36 min (conditions B).

Preparation of 1,3,5-Trimethyluracil (28). Following the procedure for preparation of 1,3-dimethyluracil (**25**), 5-methyluracil (thymine, 10.0 g, 79.4 mmol), sodium hydroxide (7.5 g, 189 mmol), and dimethyl sulfate (23.3 g, 185 mmol) yielded 7.4 g (61%) of 1,3,5-trimethyluracil (**28**), mp 135–138 °C (lit.[25] mp 153 °C).

Ozonation of 1,3,5-Trimethyluracil (28). Following the above conditions for the ozonation of 1,3-dimethyluracil (**25**), a quantity of 1,3,5-trimethyluracil (1.0 g, 6.5 mmol) was allowed to react with ozone–oxygen (600 mL/min, 17 mg of O_3/min) for 45 min. The reaction mixture was analyzed by GC/MS (conditions C) to yield **methylpyruvamide (29)** (t_r 1.5 min) and **1,3,5-trimethyl-5-hydroxyhydantoin (30)** (t_r 7.2 min).

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Supplementary Material Available: Spectroscopic data for **2–5**, **8**, **9**, **11–17**, **21**, **23–26**, and **28–30** (7 pages). Ordering information is given on any current masthead page.

Synthesis of the Dihydro Diols and Diol Epoxides of Chrysene from Chrysene

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Synthesis of the 1,2- and 3,4-trans dihydro diols (**1a** and **2a**) of chrysene and the corresponding anti isomeric diol epoxides (**3a** and **4**) is described. Synthesis of both **1a** and **2a** is accomplished in only seven steps from chrysene via initial hydrogenation over Adam's catalyst, dehydrogenation of the resulting 1,2,3,4-tetrahydrochrysene by NBS and DBN to a mixture (2:1) of 1,2- and 3,4-dihydrochrysene, Prévost reaction and chromatographic separation of isomers, followed by dehydrogenation and basic methanolysis of each isomer. An alternative regiospecific synthesis of **1a** involving initial hydrogenation of chrysene over a PtO_2–Pd/C catalyst to 1,2,3,4,5,6-hexahydrochrysene is also presented. These methods offer major advantages over established methods for the synthesis of polyarene dihydro diols which entail more complex multistep ring construction. NMR analysis indicates **1a** to exist preferentially in a diequatorial conformation, while **2a** is exclusively diaxially oriented. Epoxidation of **1a** affords stereospecifically the anti diol epoxide **3a**, whereas similar reaction of **2a** furnishes the corresponding anti and syn diol epoxides in a 5:3 ratio. Preliminary experiments indicate **3a** to be a potent inhibitor of the infectivity of ϕX174 DNA viral replication in *E. coli* spheroplasts, while **4** appears only weakly active.

Chrysene is a weak carcinogen and a widespread environmental contaminant.[1] It is present in the atmosphere, soil, marine sediments, automobile exhaust, smokestack effluents, cigarette smoke, and foods.[1,2] Recent research has implicated diol epoxide metabolites as the principal active forms of benzo[*a*]pyrene[3] and other carcinogenic polycyclic arenes.[4] Other evidence suggests that other types of active metabolites may also be involved.[5] In the case of chrysene, the 1,2- and 3,4-dihydro

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